Investigation of factors affecting the exposure of orally-administered drugs

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Abstract

In drug development, it is important to evaluate drug exposure, since efficacy and safety of a drug are related to drug exposure. Based on this concept, equivalency of drug exposure is used for demonstrating therapeutic equivalence of different formulations in bioequivalence (BE) studies, and drug-drug interaction (DDI) studies are conducted to know an effect of a co-administered drug on drug exposure of a test drug. Thus, to know factors affecting drug exposure is useful for efficient clinical development. In this research, factors affecting intra-subject variability of drug exposure and factors relating to effect of acid-reducing agents (ARAs) on drug exposure were investigated.

First, we investigated factors affecting intra-subject variability of drug exposure, which affect the results of BE studies focusing on two factors: absolute oral bioavailability (BA) and acidic nature of drugs. The relationship between absolute oral BA and intra-subject variability of drug exposure (Cmax and AUC) showed negative log-linear relationship in the BE studies of 65 orally-administered immediate-release drugs under fasted condition. Drugs with poor absolute oral BA less than 5% showed high intra-subject CV in the range of 30%-65%. In contrast, drugs with high absolute oral BA more than 80% showed low intra-subject CV less than 20%. Also, acidic drugs with pKa < 6 had higher intra-subject CV of Cmax than AUC compared to other types of drugs. The intra-subject CV ratios of Cmax to AUC for acidic drugs with pKa < 6 were significantly higher than those for other types of drugs.

Second, we investigated factors relating to an effect of acid-reducing agents (ARAs) on drug exposure. Twenty-nine DDI study results with ARAs were selected and the relationships between the effect of ARAs on Cmax or AUC ratio (with/without ARA)

and potential factors influencing drug exposure such as dose number (dose/250/solubility at neutral pH) and solubility ratio (acidic/neutral pH) were investigated. The effect of ARAs on the Cmax or AUC ratio decreased with the increasing value of both factors, but the solubility ratio was a more appropriate predictor for the effect of ARAs on drug exposure.

For factors affecting intra-subject variability of drug exposure, it would be efficient to take different strategies based on absolute oral BA values. If absolute oral BA is < 5%, high intra-subject CV is expected so that applying replicate crossover design and two-stage BE is recommended to mitigate a risk of BE failure. If absolute oral BA is > 80%, low intra-subject CV is expected, so estimating sample size by assuming the intra-subject CV of 20% would be efficient. In addition, it is recommended to confirm whether a test drug is acidic compound with pKa < 6 when planning a BE study. ARAs may affect drug exposure for orally-administered drugs exhibiting pH dependent solubility. Since solubility ratio of acidic pH to neutral pH was found to be a good predictor to estimate the effect of ARA on drug exposure, investigating solubility at acidic and neutral pH is proposed in early stage of drug development. And it is recommended considering necessity and timing for a dedicated DDI study with ARAs based on the predicted effect of ARAs on drug exposure.

The factors affecting drug exposure investigated in this research are useful for establishing an appropriate study design and determining necessity and timing of clinical studies. Thus, our findings can contribute to efficient clinical development of orally-administered drugs in the future.

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Abbreviations

Abbreviation	Definition
ADME	absorption, distribution, metabolism and excretion
ANOVA	analysis of variance
ARA	acid-reducing agent
AUC	area under the drug concentration-time curve
BA	bioavailability
BCS	biopharmaceutics classification system
BE	bioequivalence
CI	confidence interval
Cmax	maximum drug concentration
CTD	common technical document
CV	coefficient of variation
CYP	cytochrome P450
D0	dose number
DDI	drug-drug interaction
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
GI	gastrointestinal
H2B	H2 blocker
HIV	human immunodeficiency virus
HVD	highly variable drug
MSE	mean square error
OCT	organic cation transporter
PK	Pharmacokinetic(s)
pKa	acid dissociation constant
PMDA	Pharmaceuticals and Medical Devices Agency
PPI	proton pump inhibitor

1. Introduction

In drug development, drug exposure such as Cmax and AUC is evaluated in most clinical studies, and exposure-efficacy and -safety relationships are explored to select clinical dose to optimize benefit/risk balance. It is based on the concept that drug exposure is thought to be related to efficacy and safety of drugs (**Figure 1**).

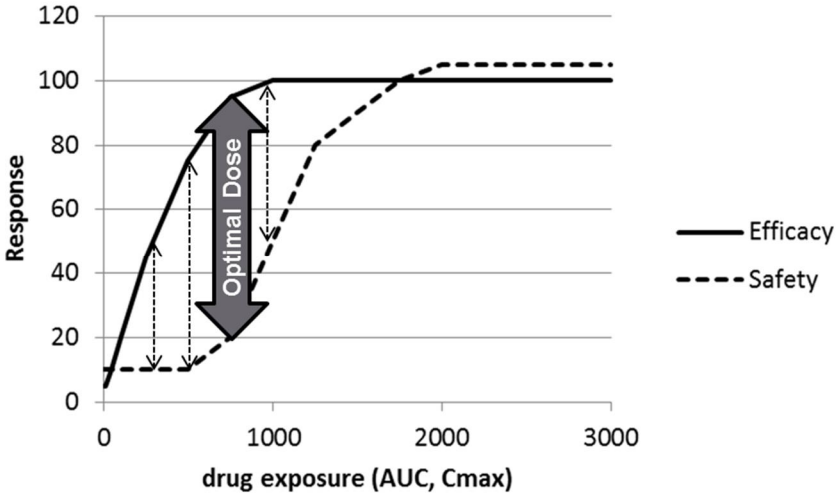


Figure 1 Relationship between drug exposure and efficacy or safety response

During clinical development for orally-administered drugs, drug formulations are commonly changed; capsule formulation used in a first-in-human study, for example, is changed to tablet formulation as a final market formulation. Sometimes formulation or manufacturing process of investigational drugs used for pivotal trials is changed in order to prepare smaller formulation for being easy to swallow or to increase manufacturing scale. When formulations are proposed to be changed, a bioequivalence (BE) study is conducted to demonstrate similarity of drug exposure between test and reference formulations. In BE studies, based on the concept that drug exposure is related to efficacy and safety of the drug, similarity of drug exposure between different

formulations is examined for ensuring therapeutic equivalency. In BE studies, it is required that 90% confidence intervals (CIs) of test/reference ratio for Cmax and AUC following drug administration fall within 80.00-125.00 percent. The 90% CI of test/reference ratio is determined by the point estimate (i.e. true ratio), number of subjects and the degree of intra-subject variability. It is useful to know the degree of intra-subject variability for drug exposure before conducting a BE study, because intra-subject variability is one of the major determinants for sample size estimation of a BE study and it is well-known that a risk of BE failure increases with the increasing intra-subject variability. In addition, for drugs having high intra-subject variability, there are some counterplans provided by health authorities to mitigate a risk of BE failures. Therefore, we investigated factors affecting intra-subject variability of drug exposure based on BE study results.

In drug-drug interaction (DDI) studies, the effect of a co-administered drug on drug exposure of a test drug is evaluated by variables of Cmax and AUC following the administration of a test drug alone or combination with a co-administered drug. It is important to assess factors affecting drug exposure in early clinical development, because there is a possibility that they may affect efficacy and safety results in pivotal clinical trials. Thus, knowledge of potential factors affecting drug exposure is useful for securing safety of patients enrolled in clinical studies and for constructing appropriate study design by managing those factors in clinical development. Also, it is important to describe the factors affecting PK in package insert to provide appropriate information about precautions to clinicians, pharmacists and patients. A DDI study with acid-reducing agents (ARAs) is one of DDI studies to assess the effect of gastric pH elevation on PK for a test drug. In recent years, the DDI study with ARAs is interested

especially in molecularly targeted anti-cancer drug development. However, there are few reports investigating predictors for the effect of ARAs on drug exposure. Thus, we investigated it based on DDI study results for orally-administered drugs with or without ARAs under fasted condition.

Recently, drug development cost is getting bigger and bigger, and efficient development is highly desirable by establishing appropriate clinical trial design and by skipping unnecessary clinical trials. Thus, we conducted the present study to investigate factors affecting drug exposure for orally-administered drugs, focusing on BE studies and DDI studies with ARAs.

This thesis consists of the following two studies:

1. Investigation of factors affecting intra-subject variability of Cmax and AUC for orally-administered drugs
2. Investigation of factors affecting Cmax and AUC for orally-administered drugs with or without ARAs.

2. Part I: Factors affecting intra-subject variability of drug exposure

2.1. Introduction

BE studies are used for assessing equivalency of drug exposure between a test and a reference product. It is thought that the PK equivalency secures therapeutic equivalency, because drug exposure is related to efficacy and safety of test drugs. Studies to establish BE between two products are important for formulation or manufacturing changes occurring during drug development and post-approval stages, in addition to registration of generic drug products. Regulatory requirements for BE are different between countries, but generally it is required that 90% CIs of test/reference ratio for Cmax and AUC following drug administration fall within 80.00-125.00 percent. The 90% CI of test/reference ratio is affected by the point estimate (i.e. true ratio), number of subjects and the degree of intra-subject variability.

For highly variable drug (HVD) products, defined as those for which intra-subject CV in Cmax and AUC is 30% or greater, a large number of subjects are needed to demonstrate BE, because high intra-subject CV extends the range of the CI. Tanguay et al. [1] examined over 1200 BE studies and observed that the failure rate increased with the increase of intra-subject variability, reaching 85% when intra-subject CV was greater than 35%. To avoid unnecessary human testing, the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) proposed a reference-scaled BE method for HVD products [2]. However, in this case, replicated crossover design is required to know the extent of intra-subject variability for a reference product. Canadian, Japanese and European BE guidelines allow to add subjects and to perform a pooled statistical analysis in case of an inconclusive outcome after an initial group of subjects

to increase the power of the study when pre-defined in the protocol [3]. However, this add-on subject study would have enormous impact on cost and timeline for drug development. Thus, it would be useful to know the extent of intra-subject variability for drug exposure before conducting a BE study. In the present study, we investigated factors affecting intra-subject variability of drug exposure based on BE study results following single oral administration of immediate-release drug products under fasted condition. We focused on two factors: absolute oral BA and acidic nature of drugs.

The first factor is the absolute oral BA. Davit et al. [4] reported that approximately 60% of HVD products were highly variable mainly due to drug substance characteristics. And it is considered that extensive first-pass metabolism and poor absorption are major factors contributing to high variability. Since both extensive first-pass metabolism and poor absorption are related to absolute oral BA, we investigated whether there is a relationship between the degree of intra-subject variability and absolute oral BA for our first objective in this study.

The second factor is acidic nature of drugs. We have observed that intra-subject CV of Cmax is considerably higher than that of AUC in acidic drugs. We thought that Cmax of acidic drugs is susceptible to gastrointestinal pH based on the simulation by Tsume et al. [5]. They simulated drug dissolution and absorption with variation in intestinal pH for BCS class 2 weak acidic drugs, and showed that gastrointestinal pH had enormous impact on the dissolution and the oral absorption. The value of Cmax was reduced with lowering gastrointestinal pH, while the AUC showed marginal change. It is because BCS class 2 weak acidic drugs act like BCS class 1 compound in the small intestine at neutral pH. For variability of physiological pH, Dressman et al. [6] reported that gastric pH in healthy subjects under fasted condition was fluctuating and elevated gastric pH

(pH above 4) was observed for average 7 min during the 60 min pH monitoring. If this elevated gastric pH occurs just after oral administration of acidic drugs, it would result in increasing the rate of dissolution of acidic drugs due to its pH-dependent solubility and, hence, lead to an increase in the rate of absorption, Cmax. Consequently, fluctuation of gastric pH under fasted condition would lead to an increase in intra-subject variability of Cmax for acidic drugs. Thus, we investigated whether acidic drugs have higher intra-subject CV of Cmax as compared to that of AUC.

Based on these hypotheses, we conducted the present study with the aim of investigating factors affecting intra-subject variability of drug exposure, focusing on absolute oral BA and acidic nature of drugs.

2.2. Method

Literature search

To obtain BE study results of immediate-release drug products after single oral administration under fasted condition, PubMed literature search was performed in 2008-2014 with the following terms: included ñbioequivalence, crossover, single, healthy and bloodò and NOT included ñextendedò. Then, inappropriate articles such as not fasting BE studies, not oral administration, and no parent drug PK data were excluded. BE data of metabolites were inappropriate for this study because metabolism is an additional factor of variability as compared to a parent drug and absolute oral BA is calculated based on AUC of a parent drug. Drugs having absolute oral BA, which was obtained from US package insert (Drugs@FDA [7]), were used for our first objective.

Also, for our second objective, test drugs were classified into four types (acidic with pKa <6, acidic with pKa Ó6, basic and zwitterionic drugs) based on functional group in its structure and pKa values. Predicted pKa values of drugs were used from chemicalize.org by ChemAxon [10]. Acidic drugs were separated by the pKa value of 6, because dissolution rate of acidic drugs with pKa <6 would be more sensitive to pH change in gastrointestinal tract following oral administration of drugs. Three neutral drugs were removed from the dataset due to limited number of drugs.

Estimation of intra-subject variability

To estimate intra-subject CV of Cmax and AUC, residual variance was estimated from the number of subjects and 90% CIs, which were obtained from each BE study by the following equation:

$90\%CI = EXP(Diff \pm t_{0.05,N-2} * SQRT(s^2 * 2/N))$,

where Diff represents the difference between the test and reference means of the logarithmically transformed metric mT and mR, s^2 is the residual (within-subject) variance of the logarithmically transformed characteristics [calculated as the mean square error (MSE) of analysis of variance (ANOVA)], and N is the number of participants in the BE study.

Then the residual variance was converted to intra-subject CV by the following equation:

$\text{Intra-subject CV} = SQRT(EXP(s^2)-1)$.

The estimated intra-subject CV values were consistent with the observed data that were calculated in some articles tested in our study (data not shown). When one compound has multiple intra-subject CV values, the geometric mean was calculated for the subsequent analysis.

Absolute oral BA

Absolute oral BA was obtained from US package insert (Drugs@FDA). If only minimum to maximum data is available, the arithmetic mean value was calculated. For clopidogrel and mycophenolate mofetil, which have extremely poor oral BA, the absolute oral BA was calculated by using AUC values following intravenous administration from articles [8, 9]. For calculation of absolute oral BA, dose-normalized

oral AUC values were divided by dose-normalized intravenous AUC values as follows:

Absolute oral BA (%) = (AUC_{oral} / $Dose_{oral}$) / ($AUC_{intravenous}$ / $Dose_{intravenous}$) x 100

Statistical analysis

Relationships between absolute oral BA and intra-subject CV of Cmax and AUC were analyzed by log-linear regression. Relationships of intra-subject CV between Cmax and AUC separated by drug type were analyzed by simple linear regression. Intra-subject CV ratios of Cmax to AUC were compared between four types of drugs (acidic with pKa < 6, acidic with pKa Ó 6, basic and zwitterionic drugs) using the Studentôs t-test (significance level of 0.0083, Bonferroni correction) using StatsDirect (ver 2.7.9; StatsDirect Ltd, Altrincham, Cheshire, UK).

2.3. Result

In the present study, 1) relationships between intra-subject CV and absolute oral BA, and 2) relationships between intra-subject CV of Cmax and AUC classified by drug type were investigated based on the articles obtained through the literature search. **Figure 2** shows the flowchart of the literature search in the study.

Based on the PubMed search as shown in section 2.2, 65 drugs with 129 articles were selected as Dataset 1. **Table 1** shows the list of compounds for Dataset 1 with absolute oral BA, which are represented in parenthesis. Also, 137 drugs with 235 articles were selected as Dataset 2 and classified into 4 types based on functional group and pKa values as acidic (pKa < 6), acidic (pKa Ó 6), basic and zwitterionic drugs. **Table 2** shows the list of acidic drugs with pKa < 6 selected from Dataset 2, with acidic functional group, pKa and intra-subject CV values.

Figure 3 shows the relationship between absolute oral BA and intra-subject CV of AUC and Cmax based on the data from Dataset 1. Intra-subject CV increased with a decrease in absolute oral BA. When absolute oral BE was less than 5%, the intra-subject CV values ranged from 30% to 48% for AUC and 30% to 65% for Cmax, which are regarded as HVD products in BE guidelines. On the other hand, when absolute oral BE was more than 80%, the intra-subject CV values of Cmax and AUC were less than 20%, except for one case (moxifloxacin: CV of Cmax was 28%). Relationship between oral BA and intra-subject CV showed negative correlation. The R^2 values in the log-linear regression for AUC and Cmax were 0.703 and 0.517, respectively.

Figure 4 shows the relationship of intra-subject CV between Cmax and AUC by acidity. Each type of drugs showed linear positive correlation with R^2 values ranging from

0.626 to 0.763. Acidic drugs with pKa < 6 showed the highest slope of the regression line (approximately 2.52) and slopes for other types of drugs ranged from 0.75 to 1.15. Mean ± standard deviation values for intra-subject CV ratios of Cmax to AUC were 2.00 ± 0.64 for acidic drugs with pKa < 6, 1.41 ± 0.59 for acidic drugs with pKa Ó 6, 1.41 ± 0.50 for basic drugs and 1.38 ± 0.59 for zwitterionic drugs. **Figure 5** shows box-and-wisker plot for each type of drugs. The ratio for acidic drugs with pKa < 6 was significantly higher than that for any other types with P values less than 0.0083.

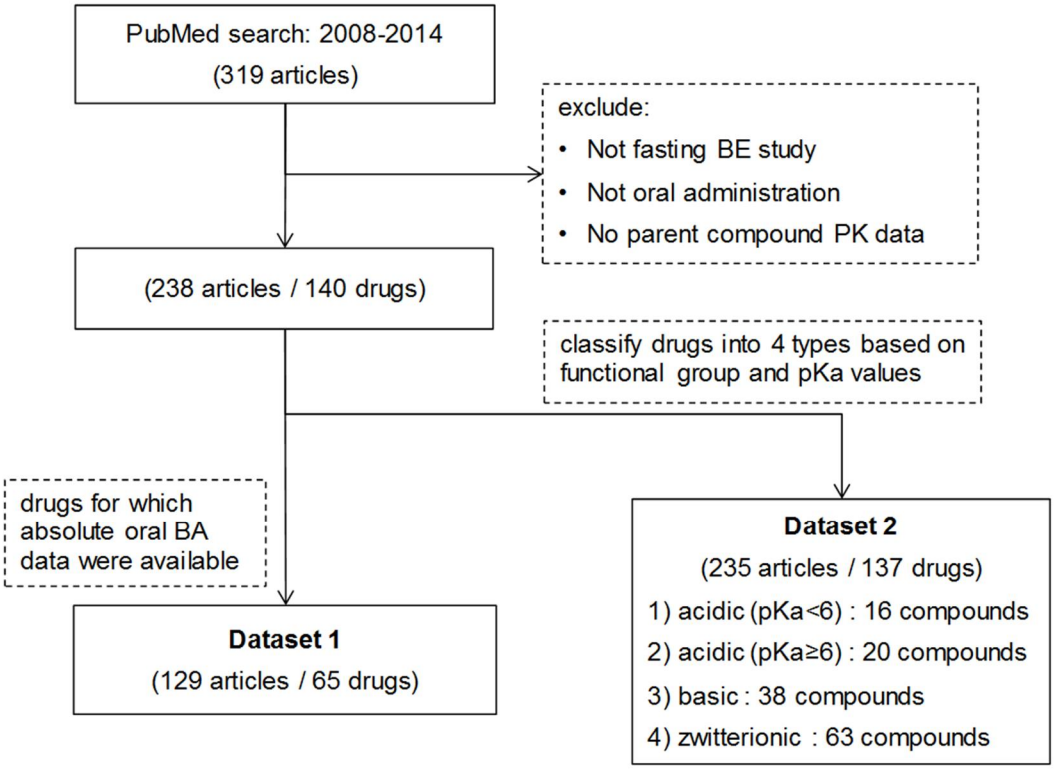


Figure 2 Flowchart of the Literature Search for this study

BA: bioavailability, BE: bioequivalence, PK: pharmacokinetics

Table 1 List of drugs with absolute oral BA in Dataset 1

Drug Name	aBA	Drug Name	aBA	Drug Name	aBA
Abacavir	85	Ethinylestradiol	55	Nateglinide	73
Alendronate	0.62	Finasteride	63	Nevirapine	93
Aliskiren	2.5	Gabapentin	60	Ondansetron	56
Ambroxol	75	Hydrochlorothiazide	71	Pitavastatin	51
Amlodipine	77	Ibandronate	0.6	Pregabalin	90
Atomoxetin	78.5	Imatinib	98	Ramipril	28
Atorvastatin	14	Irbesartan	70	Repaglinide	56
Azithromycin	38	Ketorolac	100	Risperidone	70
Bisoprolol	80	Lamivudine	86	Rosiglitazone	99
Bosentan	50	Lamotrigine	98	Rosuvastatin	20
Cefdinir	18.5	Levetriacetam	100	Saquinavir	4
Ciprofloxacin	70	Levofloxacin	99	Sildenafil	41
Clonidine	75	Losartan	33	Sirolimus	17.8
Clopidogrel	0.208*	Lovastatin	5	Stavudine	86.4
Cyclobenzaprin	44	Meloxicam	89	Tacrolimus	18
Darunavir	37	Metformin	55	Tizanidine	40
Diclofenac	50	Metoclopramide	80	Tramadol	75
Digoxin	70	Mirtazapine	50	Trospium	9.6
Drospirenone	80.5	Montelukast	64	Valproate	100
Erlotinib	60	Moxifloxacin	90	Valsartan	25
Eplerenone	69	Mycophenolate mofetil	0.502*	Zidovudine	64
Escitalopram	80	Naproxen	95		

Values in parenthesis represent absolute oral BA (%).

aBA: absolute oral BA

* Absolute oral BA was calculated by dividing dose-normalized AUC_{oral} by dose-normalized $AUC_{intravenous}$; AUC_{oral} was derived from literature used in Dataset 1 and $AUC_{intravenous}$ was derived from literatures for clopidogrel [8] and mycophenolate mofetil [9], respectively.

Table 2 List of acidic drugs with pKa less than 6 in Dataset 2

Drug name	Acidic functional	pKa [a]	Intra-subject CV(%) [b]		
	group		Cmax	AUC	Ratio (Cmax/AUC)
Aceclofenac	carboxylic acid	3.4	25	10	2.4
Artesunate	carboxylic acid	3.8	58	19	3.1
Atorvastatin	carboxylic acid	4.3	32	15	2.2
Bosentan	sulfonamide	5.8	39	17	2.3
Diclofenac	carboxylic acid	4.0	39	16	2.5
Flurbiprofen	carboxylic acid	4.4	15	9	1.7
Glimepiride	sulfonylurea	4.3	14	11	1.3
Ibuprofen	carboxylic acid	4.9	21	6	3.3
Isotretinoin	carboxylic acid	5.0	9	7	1.3
Ketorolac	carboxylic acid	3.8	19	8	2.4
Naproxen	carboxylic acid	4.2	12	6	1.9
Nateglinide	carboxylic acid	4.0	17	9	1.8
Probenecid	carboxylic acid	3.5	17	10	1.7
Rosuvastatin	carboxylic acid	4.0	22	17	1.3
Triflusal	carboxylic acid	3.4	17	12	1.4
Valproic acid	carboxylic acid	5.1	15	11	1.4

a) Predicted values derived from ChemAxon website (http://www.chemicalize.org/) [10]

b) Intra-subject CV was estimated in this study (Methods section)

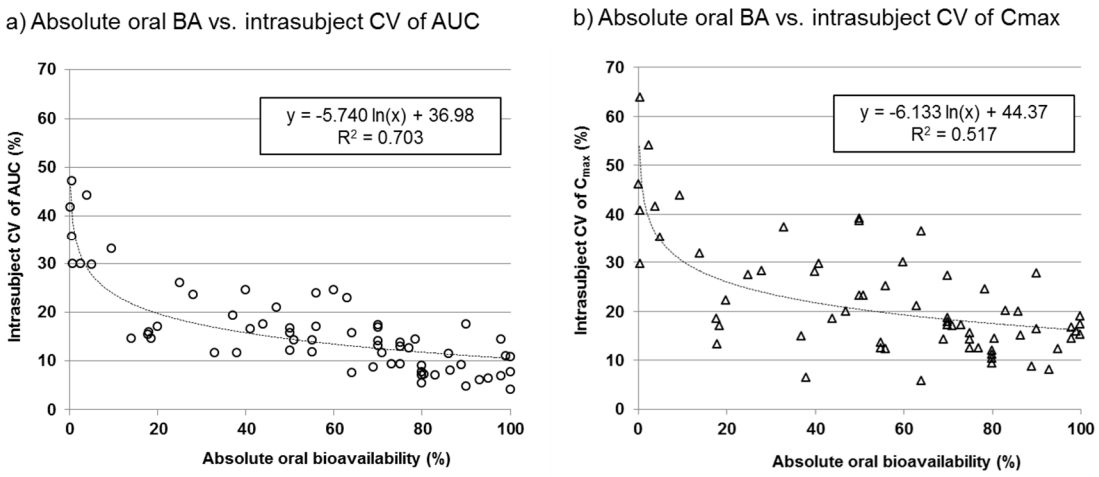


Figure 3 Relationship between absolute oral BA and intrasubject CV of AUC or Cmax

Open circle (ʒ, n=65) and triangle (æ, n=65) represent data for intrasubject CV of AUC and Cmax, respectively. Dotted line shows log-linear regression between absolute oral BA and intrasubject CV. The regression equation and coefficient of determination (R^2) are shown in the box.

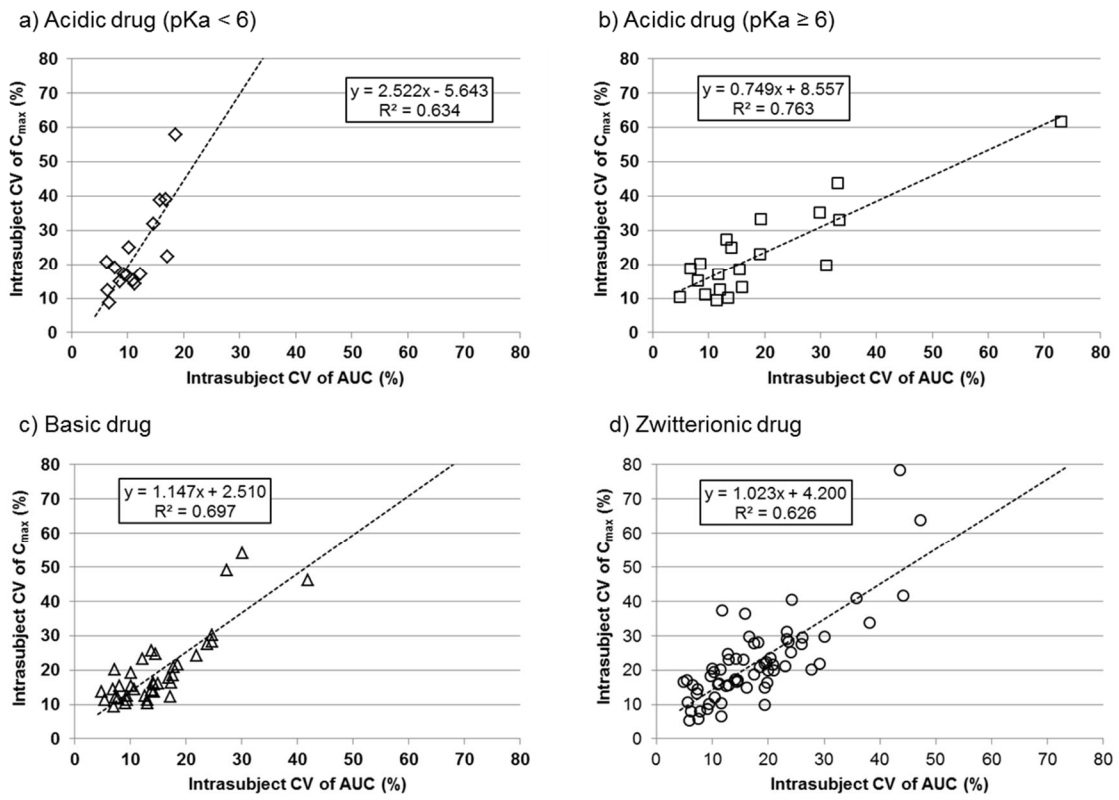


Figure 4 Relationship of intrasubject CV between Cmax and AUC by acidity [acidic (pKa < 6 and pKa ≥ 6), basic and zwitterionic drugs]

Open diamond (N=16), square (N=20), triangle (N=38) and circle (N=63) represent acidic drug with pKa <6, acidic drug with pKa Ó 6, basic drug and zwitterion drug, respectively. Dotted line shows simple linear regression of intrasubject CV between Cmax and AUC. The regression equation and coefficient of determination (R^2) are shown in the box.

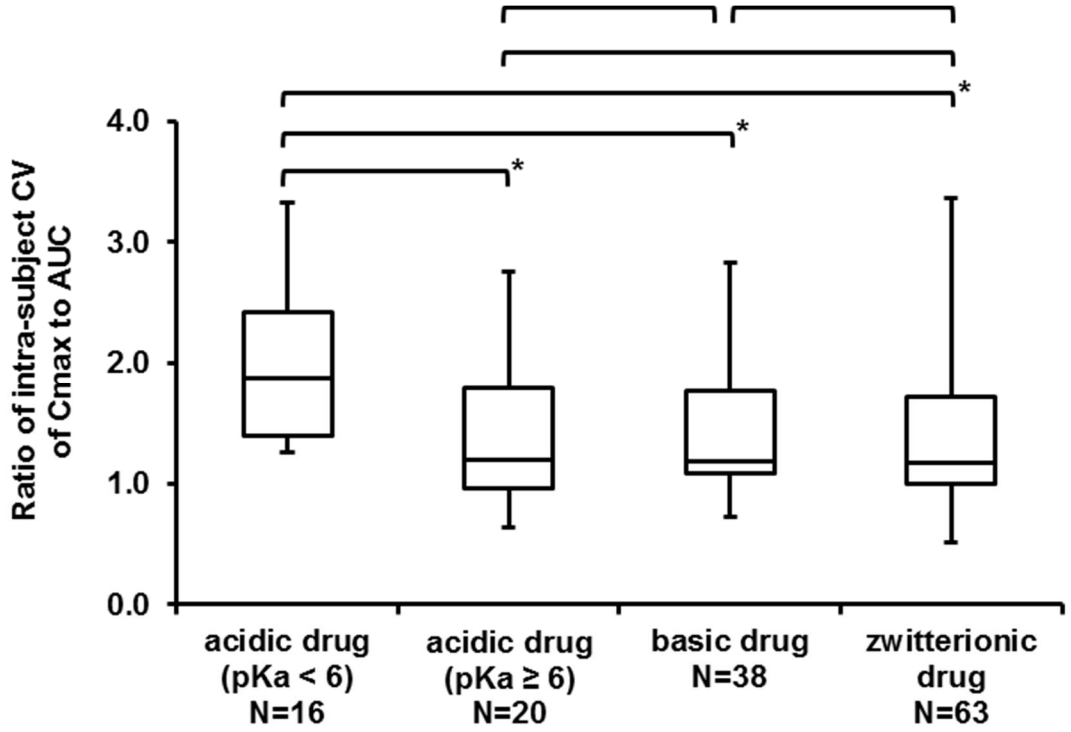


Figure 5 Box-and-whisker plot for ratio of intra-subject CV of Cmax to AUC by drug types

*: Statistically significant difference observed after Bonferroni correction ($p < 0.0083$)

2.4. Discussion

In the present study, we investigated factors affecting intra-subject variability of drug exposure: absolute oral BA and acidic nature of drugs. The relationship between absolute oral BA and intra-subject variability of drug exposure (Cmax or AUC) showed roughly log-linear negative correlation (**Figure 3**) and the intra-subject variability increased with a decrease in absolute oral BA. Concerning the difference of intra-subject variability between acidic drugs and other types of drugs, the values of intra-subject CV ratios of Cmax to AUC for acidic drugs were significantly higher than those for other types.

Davit et al. [4] reviewed over 1000 BE studies of 180 different drugs evaluated by FDAâs Office of Generic Drugs (OGD) during 2003-2005, of which 31% were highly variable for Cmax or AUC. Approximately 60% of the HVDs were due to drug substance pharmacokinetic characteristics, and formulation performance contributed to the high variability for only about 20% of the total. They also studied drug substance characteristics contributing to high variability and addressed that 83% (29 out of 35 drugs) of HVDs were subject to extensive first-pass metabolism and the others were of low aqueous solubility (skeletal muscle relaxant), acid labile (reverse transcriptase inhibitor), and low oral BA (bisphosphonates). Since low aqueous solubility leads to poor oral absorption and bisphosphonates are known to be poor oral absorption due to their very low lipophilicity and ionized form at physiological pH [11], it is considered that poor absorption is another major factor contributing to high variability. After oral administration of drug products, systemic drug exposure is affected by absorption and first-pass metabolism. Thus, it is thought that absolute oral BA, which is derived from fraction of absorption and fraction of availability passing pre-systemic metabolism,

seems one of the major factors affecting the extent of intra-subject variability.

As shown in **Figure 3**, intra-subject CV of AUC and Cmax increased with a decrease in absolute oral BA. As far as we know, this is the first research that systematically investigates the relationship between absolute oral BA and intra-subject variability of drug exposure. The relationship between intra-subject CV and absolute oral BA are characterized by log-linear regression. In our results the R^2 value for AUC was higher than that for Cmax. It would be reasonable, since absolute oral BA values are calculated based on AUC values from oral and intravenous administration.

In drug development, a relative or formal BE study is occasionally required without prior information of intra-subject CV. Based on our results, intra-subject CV of AUC and Cmax were generally less than 20% when absolute oral BA is more than 80%. To facilitate drug development for drugs with high absolute oral BA, it would be one of the options to plan a BE study setting 20% for intra-subject CV of sample size estimation, without doing a pilot study for assessing intra-subject CV. In contrast, when absolute oral BA is less than 5%, intra-subject CV showed more than 30%, which is regarded as HVDs. Thus, for drugs with low absolute oral BA, it is better to plan a BE study with replicated crossover design. Also, two-stage BE approach, which needs to describe the possibility of adding subjects in the protocol based on the first BE test, would be useful to mitigate a risk of failure from the first BE test.

We also examined whether acidic nature of drugs tend to be affiliated with much higher intra-subject variability of Cmax as compared to that of AUC. The slope of intra-subject CV of Cmax to that of AUC for acidic drugs with pKa < 6 showed 2.52, which is higher than other types of drugs (**Figure 4**). In general, intra-subject CV of Cmax is slightly

higher than that of AUC, since it would be difficult to obtain true Cmax values in clinical studies. However, the relatively high slope for acidic drug with pKa <6 cannot be explained only by general difference of intra-subject CV between Cmax and AUC. It was further confirmed by investigating intra-subject CV data derived from other acidic drugs of which pKa is less than 6: acetylsalicylic acid (intra-subject CV 24% for Cmax, 18% for AUC [12]), captopril (31% for Cmax, 16% for AUC [12]), furosemide (60% for Cmax, 22% for AUC [12]), gemfibrozil (32% for Cmax, 12% for AUC [12]), glibenclamide (22% for Cmax, 14% for AUC [13]) and mefenamic acid (48% for Cmax, 15% for AUC [14]). These additional data were consistent with the results in Figure 3-a).

Considering that acidic drugs with pKa < 6 have much higher intra-subject CV of Cmax than that of AUC, we also tested whether the R^2 value for the relationship between absolute oral BA and intra-subject CV of Cmax from Dataset 1 is improved when data from such drugs are excluded. The R^2 value was increased from 0.517 to 0.560 when the data from atorvastatin, bosentan, diclofenac, ketorolac, naproxen, nateglinide and valproate were excluded.

Generally, acidic drugs having carboxyl group such as non-steroidal anti-inflammatory drugs (NSAIDs) have poor solubility at low pH and high solubility at neutral pH, which depends on fraction of ionic form of a compound. This pH-dependent solubility has also an impact on dissolution profile of drug products. Tsume et al. [5] simulated drug dissolution and absorption for BCS class 2 weak acidic drugs (ibuprofen and ketoprofen) when gastrointestinal pH is lowered. Gastrointestinal pH had enormous impact on the dissolution and, hence, the oral absorption of ibuprofen. When the pH was lowered by 2.0 compared with the average physiological pH range in human (pH

6.0-7.4), the value of Cmax was reduced by 58.1%, while the AUC showed marginal change. The reason why AUC showed marginal change is because BCS class 2 weak acidic drugs act like BCS class 1 drug in the small intestine at neutral pH. Rinaki et al. [15] also suggested that BCS classification of NSAIDs, most of which are acidic drugs with extensive absorption, move from Class 2 to Class 1 as pH increases from 1.2 to 7.4, by using a quantitative version of BCS.

Dressman et al. [6] investigated gastric pH in healthy subjects under fasted condition. Their data indicated that the gastric pH was fluctuating and elevated to higher values (pH above 4) in most subjects (average duration, 7 ± 6 min during 60 min monitoring). Therefore, there is a possibility that solubility of acidic drugs is elevated at increased pH and its absorption rate increases when the elevation of pH occurs close to the timing of drug administration. Since it is thought that increased pH occurs unexpectedly, this then leads to an increase in variation of drug exposure, especially in Cmax based on pH-dependent solubility of acidic drugs

In fact, BE study results from enteric-coated tablets of diclofenac, which is an acidic drug with pKa 4.0, showed that the intra-subject CV of Cmax and AUC were estimated to be 13% and 13%, respectively [16]. In contrast, intra-subject CV values for immediate-release tablets of diclofenac in this study were 39% and 16% for Cmax and AUC, respectively. In enteric-coated formulation, impact of gastric pH fluctuation would be masked because dissolution occurred only at higher pH range in gastrointestinal tract. These observations indicate that enteric-coated formulation has a potential to reduce intra-subject CV of Cmax for acidic drugs as compared to immediate-release formulation.

Extensions for biowaivers of BCS class 2 compounds are under discussion based on simulation results from several groups [15, 17, 18, 19]. Generally they argued that some BCS class 2 acidic drugs are justified to be subject to a biowaiver, because these drugs can dissolve quickly and behave like BCS class 1 drugs at intestinal pH (6.5-7.0) in the gastrointestinal tract, even though they exhibit low solubility at acidic pH. However, our results indicated that acidic drugs tend to have much higher intra-subject CV of Cmax than AUC. Thus, impact of Cmax variability on efficacy and safety has to be assured when considering extension for biowaiver of BCS class 2 acidic drugs.

To investigate relationship between intra-subject CV and absolute oral BA without other influential factors, BE results under fed condition and those with controlled-release drug products were excluded from this study. However, some extent of variability for intra-subject CV still remains especially in Cmax even if drugs have similar absolute oral BA. This variability would be due to formulation effect of immediate-release drug products (e.g. variability of dissolution), dose-dependency on absolute oral BA (i.e. non-linear pharmacokinetics), physiological factors like gastric emptying, and assay variability for quantification of drug concentration. To improve predictability of intra-subject variability, further investigation is needed with consideration for these factors.

3. Part II: Factors affecting drug exposure with or without ARAs

3.1. Introduction

There are three types of acid-reducing agents (ARAs) based on the mechanism of action: antacids, histamine H2-receptor antagonists (H2 blockers), and proton pump inhibitors (PPIs). Antacids such as magnesium hydroxide exert their effects by directly neutralizing gastric acid, resulting in a quick onset and a short duration of suppression on gastric acid production. H2 blockers compete reversibly with histamine at the H2 receptors in the gastric parietal cells, leading to reduced gastric acid production. On the other hand, PPIs inactivate the proton pump (i.e., the gastric H^+, K^+-ATPase) at the secretory surface of the gastric parietal cells by irreversibly binding to the pump, resulting in suppression of gastric acid production. [20]. It was reported that ARAs were frequently administered to HIV patients (approximately 37% of the patients) and cancer patients (approximately 20%-33% of the patients) [21, 22].

A DDI study with acid-reducing agents (ARAs) is one of DDI studies to assess the effect of gastric pH elevation on PK of a test drug. For a drug having pH-dependent solubility, elevation of gastric pH by ARAs would affect fraction of ionized form and dissolution in gastrointestinal tract. Therefore, elevation of gastric pH may affect its absorption, leading to change of drug exposure.

In 2012 Budha et al. [23] reported that most molecular targeted anticancer drugs are weak bases that exhibit pH-dependent solubility, and suppression of gastric acidity with ARAs could impair their absorption and may lead to consequent reduction in efficacy. After this publication, health authorities have become liable to raise a question whether ARAs affect drug exposure of a test drug with pH-dependent solubility. And health

authorities request a dedicated DDI study with ARAs for molecularly targeted anti-cancer basic drugs having pH-dependent solubility at the post-marketing stage. However, some drugs (cabozantinib, crizotinib etc) did not show decrease of drug exposure when co-administered with ARAs in dedicated DDI studies conducted post-market, even if the test drug is a weak basic drug with pH-dependent solubility.

So far, few reports have been published in which factors affecting drug exposure with or without ARAs were investigated. Zhang et al. [20] investigated orally-administered 19 basic drugs selected from US package insert regarding effect of ARAs on PK and physicochemical properties. They proposed a preliminary conceptual framework whether or not a dedicated DDI study with ARAs for a test drug should be considered, if a test drug meets all of the following conditions: 1) basic drug, 2) pH-dependent solubility, 3) if clinical dose divided by water volume (250 mL), which is a general volume to receive an orally-administered drug, is higher than the solubility of a test drug at neutral pH. Based on their research, approximately 16% and 52% of test drugs were false positive when the criteria of positive ARA effect was defined as 25% and 50% decrease of drug exposure, respectively. Considering relatively high false positive rate (~50%) in the proposed framework, it would be desirable to find more appropriate factors affecting drug exposure following oral drug administration with or without ARAs.

To find more appropriate factors affecting drug exposure with or without ARAs, we investigated the relationship between effect of ARA on drug exposure and ratios of solubility at acidic pH to that at neutral pH as well as dose number used for the previous research.

3.2. Method

Selection of clinical data

PubMed literature search was performed from 2007 to 2015 Sep based on the following terms: (a) ñacid reducing agentò or ñproton pump inhibitorò or ñH2 blockerò, (b) ñeffectò or ñinfluenceò or ñdrug interactionò, (c) ñhumanò or ñclinical studyò, and (d) ñpharmacokineticsò. Further it was confirmed whether there are clinical results for DDI with ARAs available from US package inserts to obtain the results for anti-cancer drugs as much as possible, since DDI with ARAs has attracted attention in oncology drug development.

Some PPIs and H2 blockers are known to be substrates and inhibitors for CYP2C19 and organic cation transporter 2 (OCT2), respectively. Thus, if DDI studies with ARAs were conducted to assess drug interaction via CYP enzymes and transporters, those clinical data were excluded.

Physicochemical properties

For the selected drugs, information on physicochemical properties for solubility at acidic and neutral pH, pKa, BCS class and acidity was obtained from the publication, FDA review report and Japanese interview form for each drug.

Relationship between effect of ARAs on drug exposure and physicochemical properties

Effect of ARAs on drug exposure was evaluated by exposure ratios (AUC or Cmax values with ARAs divided by those without ARAs). When one compound has multiple

results from more than one clinical DDI studies, arithmetic mean for the exposure ratios was calculated for the subsequent analysis.

The relationship between physicochemical properties and exposure ratios were investigated. We examined mainly the following two variables as potential factors related to exposure ratios:

1. Dose number = dose tested in clinical study (mg) / 250 (mL) / solubility at neutral pH (mg/mL)
2. Solubility ratio = solubility at acidic pH (mg/mL) / solubility at neutral pH (mg/mL)

We assumed that the pH range of solubility at acidic and neutral pH were generally 1-2 and 5-7, respectively, based on the consideration for normal gastric pH under fasted condition and elevated gastric pH following ARAs under fasted condition.

3.3. Result

Selection of clinical data

Totally 29 DDI study results from 24 drugs with solubility data were obtained (**Figure 6**), which included 17 drugs co-administered with PPIs and 12 drugs co-administered with H2 blockers. Most of them were basic drugs. Summarized data of the drugs were listed in **Table 3**.

Since clopidogrel is a substrate of CYP2C19, most clinical data obtained under the condition co-administered with PPIs were excluded. However, the DDI study results of clopidogrel co-administered with rabeprazole was not excluded, because rabeprazole is thought to be a very weak CYP2C19 inhibitor [24, 25]. Cimetidine (H2 blocker) is known to be a substrate and an inhibitor for organic cation transporter 2 (OCT2) [26], and the DDI study results with cimetidine via OCT2 were excluded.

Gabapentin is freely soluble in water as well as in both basic and acidic aqueous solutions, so the solubility ratio was regarded as 1. The solubility of GSK1349572 does not change over the physiological pH range [27], so the solubility ratio was regarded as 1. The solubility data for imatinib shows that it is freely soluble (100-1,000 mg/mL) up to pH 5.5 and its pKa is 7.7, so the solubility ratio was regarded as 1. The solubility of indinavir is pH-dependent, being greater than 100 mg/mL at pH 3.0 and smaller than 0.03 mg/mL at pH 6.0. So the solubility ratio was regarded as 3333. Although the acidic pH of pH 3 is slightly higher than normal gastric pH of pH 1-2, the solubility at pH 1-2 is thought to be similar to that at pH 3 since the pKa value is 6.2. Since the solubility data for nilotinib shows that it is slightly soluble (1-10 mg/mL) at pH 1.0, very slightly soluble (0.1-1 mg/mL) at pH 2.0 and pH 3.0, and practically insoluble (<0.1 mg/mL) in buffer solutions of pH Ó4.5, the solubility ratio was regarded as 100 (10 mg/mL divided

by 0.1 mg/mL). Since revexepride has high solubility across the gastric pH range, effect on its PK due to a change in gastric pH is unlikely [28]. Thus, the solubility ratio was regarded as 1.

Relationship between Cmax or AUC ratios and dose number

In the previous research by Zhang et al. [20], dose number at neutral pH was proposed to be used as one of the criterion for selecting drugs for which the sponsor should consider conducting a dedicated DDI study with ARAs. Thus, relationship between Cmax or AUC ratios and dose number was investigated (**Figure 7**). The AUC and Cmax ratios were decreased with the increasing dose number and the relationship was described by negative log-linear regression with R^2 values of 0.47 and 0.56, respectively.

Similar to the previous research, approximately 50% of the results from this study (14 out of 29 for AUC and 10 out of 29 for Cmax) were false positives when DDI positive is defined as values with more than 50% of decrease of AUC or Cmax.

Relationship between Cmax or AUC ratios and solubility ratios (acidic/neutral pH)

The relationship between Cmax or AUC ratios and solubility ratios (at acidic pH/at neutral pH) were investigated (**Figure 8**). The AUC and Cmax ratios were decreased with the increasing solubility ratios and the relationship was well characterized by negative log-linear regression with R^2 values of 0.77 and 0.76, respectively. Drug exposure under the condition of being co-administered with ARAs seemed to be decreased by approximately 40%-50% as compared to that without ARAs when the solubility ratio is greater than 1000.

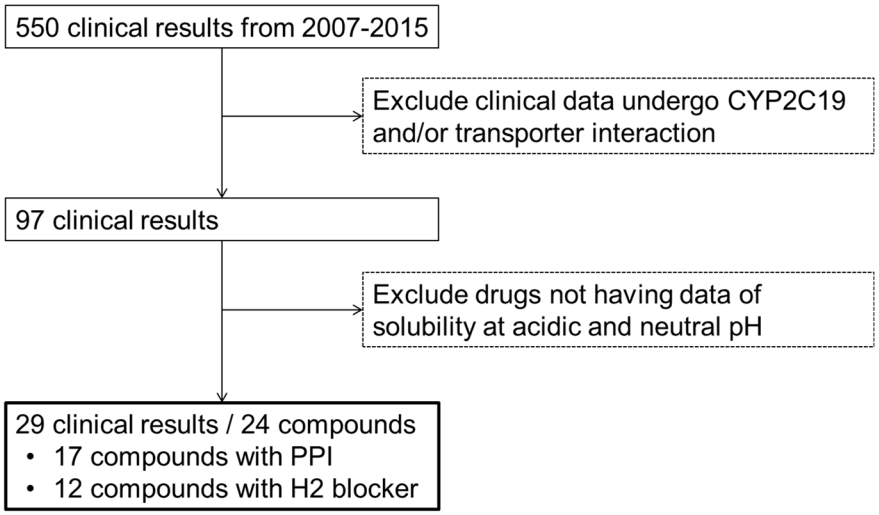


Figure 6 Selection of clinical data for this study

Table 3 List of test drugs with dose number (D0), solubility ratio (acidic/neutral pH), type of ARA, Cmax or AUC ratio% (with/without ARA)

Compound Name; [acidity]	Dose (mg)	Solubility; [Source]	D0 at neutral pH	Solubility ratio (acidic/neutral pH)	pKa	BCS class	ARA (1=PPI, 2=H2B)	Cmax ratio% (w /wo ARA)	AUC ratio% (w/wo ARA)
Alogliptin; [basic]	100	51.9 mg/mL (0.1N HCl), 21.3 mg/mL (pH 7); [Alogliptin J-IF]	0.0188	2	8.5	I	2	105	107
ARRY-403; [basic]	100	1.8 mg/mL (pH 2), 0.007 mg/mL (pH 6.3); [29]	229	257	3.9, 9.8	II	2	66	73
Atazanavir; [basic]	300	5.2 mg/mL (pH 1.9), 0.002 mg/mL (pH 5.4 and pH 8.7); [Atazanavir J-IF]	600	2600	4.7	II	1	32	31
Atazanavir	300	-	600	2600	-	-	2	63	65
Axitinib; [basic]	100	0.075 mg/mL (pH 2.2), 0.0002 mg/mL (pH 6.0); [FDA review report 2.5.1]	2000	375	4.8	II	1	58	85
Bosutinib; [basic]	400	9.4 mg/mL (pH 2), 0.02 mg/mL (pH 6.8); [FDA review report 2.5.1]	8	470	7.9	IV	1	54	74
Clopidogrel; [basic]	75	694.5 mg/mL (pH 1.2), 12.8 mg/mL (pH 6.8); [30]	0.0234	54	4.5	II	1	72	88
Clopidogrel	600	-	0.188	54	-	-	2	90	93

Compound Name; [acidity]	Dose (mg)	Solubility; [Source]	D0 at neutral pH	Solubility ratio (acidic/neutral pH)	pKa	BCS class	ARA (1=PPI, 2=H2B)	Cmax ratio% (w /wo ARA)	AUC ratio% (w/wo ARA)
Cobimetinib; [basic]	20	2.71 mg/mL (pH 2), 0.55 mg/mL (pH 7.5); [31]	0.145	5	8.85	NA	1	100	111
Dasatinib; [basic]	50	18.4 mg/mL (pH 2.6), 0.008 mg/mL (pH 6.0); [EMEA review report 2006]	25	2300	3.1, 6.8, 10.8	II	1	58	57
Dasatinib	50	-	25	2300	-	-	2	37	40
Domperidone; [basic]	10	0.5668 mg/mL (pH 1.0), 0.2433 mg/mL (pH 5); [32]	0.1644	2	7.9	II	1	84	93
Gabapentin; [zwitterionic]	1200	Freely soluble in water and both basic and acidic aqueous solutions; [USPI]	0.048 (estimated as 100 mg/mL based on freely soluble)	1	3.68 (acid), 10.70 (base)	III	2	96	99
GDC-0941; [basic]	40	0.75 mg/mL (pH 1), <0.001 mg/mL (pH6.8); [33]	160	750	1.54, 4.24	II	1	31	46
Gefitinib; [basic]	250	21 mg/mL (pH 1.0), <0.001 mg/mL (pH 7); [2012 Budha NR]	1000	21000	5.4, 7.2	II	2	30	56
GSK1322322; [unknown]	1000	1 mg/mL (pH 7); [34]	4	NA	NA	NA	2	42	62
GSK1349572; [unknown]	50	Solubility does not change over the physiological pH	NA	1	NA	II	1	92	100

Compound Name; [acidity]	Dose (mg)	Solubility; [Source]	D0 at neutral pH	Solubility ratio (acidic/neutral pH)	pKa	BCS class	ARA (1=PPI, 2=H2B)	Cmax ratio% (w /wo ARA)	AUC ratio% (w/wo ARA)
		range; [27]							
Imatinib; [basic]	400	Freely soluble (100-1,000 mg/mL) up to pH 5.5; [23]	0.016	1	7.7	NA	1	97	107
Indinavir; [basic]	800	>100 mg/mL (pH 3.0), <0.03 mg/mL (pH 6.0); [35]	107	3333	6.2	IV or II	1	59	53
Itraconazole; [basic]	200	0.004 mg/mL (pH 1), 0.000001 mg/mL (neutral pH); [36]	800000	4000	3.7	II	2	47	49
Lapatinib; [basic]	1250	0.001 mg/mL (pH 1), 0.000005 mg/mL (pH 6); [Lapatinib J-IF]	1000000	200	NA	II	1		74
Mycophenola -te mofetil; [basic]	2200	4.075 mg/mL (pH 2), 0.04 mg/mL (pH 7.0); [37]	220	102	5.6, 8.5	II	1	69	75
Nilotinib; [basic]	400	Slightly soluble (1-10 mg/mL) at pH 1.0, very slightly soluble (0.1-1 mg/mL) at pH 2.0 and pH 3.0, and practically insoluble (<0.1 mg/mL) in buffer solutions of pH Ó4.5; [23]	16	100	2.1, 5.4	IV	1	73	66
Nilotinib	400	-	16	100	-	-	2	97	91

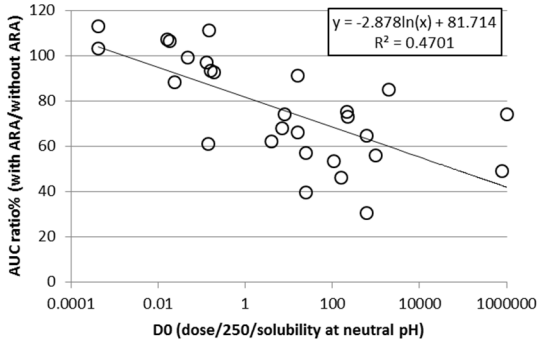
Compound Name; [acidity]	Dose (mg)	Solubility; [Source]	D0 at neutral pH	Solubility ratio (acidic/neutral pH)	pKa	BCS class	ARA (1=PPI, 2=H2B)	Cmax ratio% (w /wo ARA)	AUC ratio% (w/wo ARA)
Posaconazole; [basic]	400	617 mg/mL (pH 1.2), 5.61 mg/mL (pH 6.5); estimated graphical data from [38]	7.13	110	3.6, 4.6	II	1	54	68
Posaconazole	200	-	0.143	110	-	-	2	61	61
Revexepride; [basic]	1	High solubility across the gastric pH range, so an effect on its PK due to a change in gastric pH is unlikely; [28]	NA	1	NA	NA	1	103	104
Saxagliptin; [basic]	5	103.5 mg/mL (pH 0.7), 149.2 mg/mL (pH 5.9); [FDA review report]	0.000426	0.69	7.3	III	1	98	113
Saxagliptin	5	-	0.000426	0.69	-	-	2	114	103
Vardenafil; [basic]	10	26 mg/mL (0.01 N HCl), 0.31 mg/mL (pH 6); [Vardenafil J-IF]	0.129	84	8.8	II	2	94	97

ARA: acid-reducing agent, PPI: proton pump inhibitor, H2B: H2 blocker, BCS: Biopharmaceutics Classification System, J-IF: Japanese interview form, USPI: US package insert

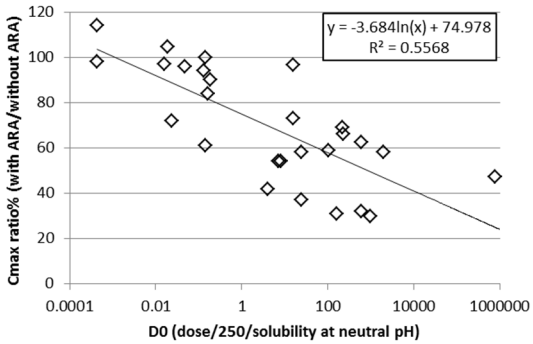
NA: not assessed

<Logarithmic scale>

a) D0 and AUC ratio%

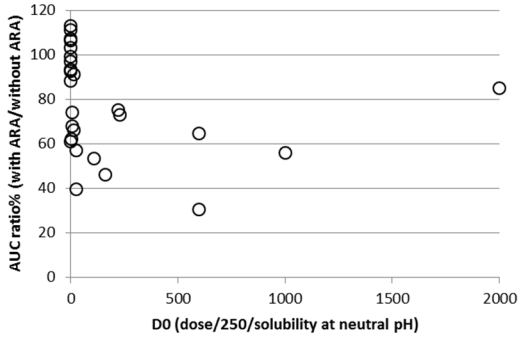


b) D0 and Cmax ratio%



<Normal scale (dose number up to 2000)>

a) D0 and AUC ratio%



b) D0 and Cmax ratio%

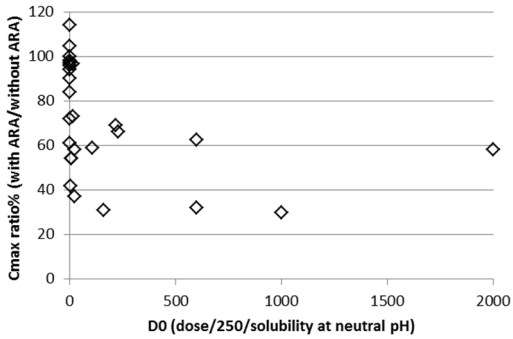
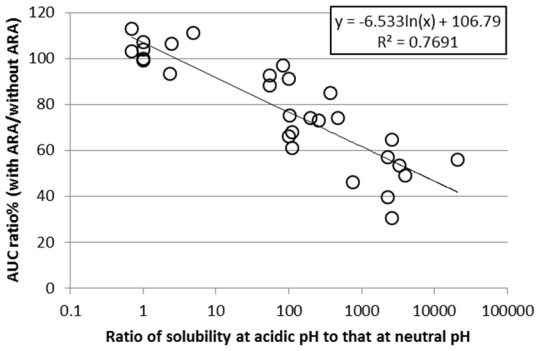


Figure 7 Relationship between dose number at neutral pH and AUC or Cmax ratio% (with/without ARA)

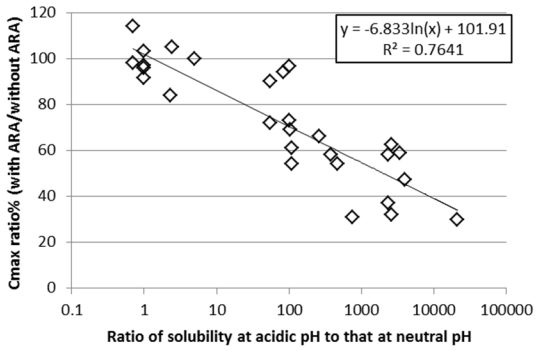
Open circle (ʒ, N=27) and diamonds (ö, N=26) represents AUC and Cmax ratio% (with/without ARA), respectively.

<Logarithmic scale>

a) Solubility ratio and AUC ratio%

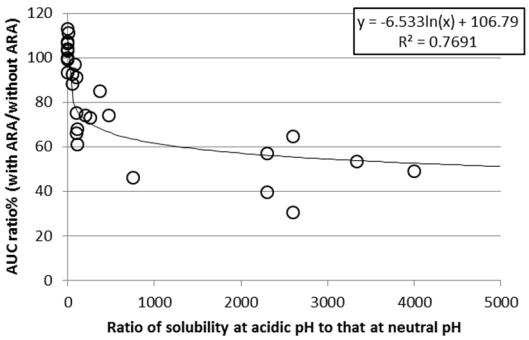


b) Solubility ratio and Cmax ratio%



<Normal scale (ratio of solubility up to 5000)>

a) Solubility ratio and AUC ratio%



b) Solubility ratio and Cmax ratio%

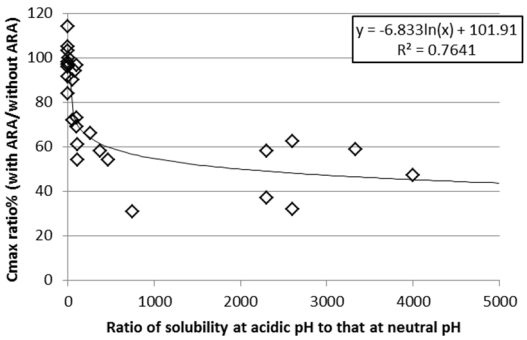


Figure 8 Relationship between solubility ratio (acidic/neutral pH) and AUC or Cmax ratio% (with/without ARA)

Open circle (ʒ, N=28) and diamonds (ö, N=27) represents AUC and Cmax ratio% (with/without ARA), respectively.

3.4. Discussion

Orally-administered drug products must undergo dissolution in the gastrointestinal (GI) tract before they can be absorbed [39]. In general, the dissolution rate (DR) is described with the following modification of the Noyes-Whitney equation [39, 40, 41]:

$$\mathrm{DR} = \frac{\mathrm{dm}}{\mathrm{dt}} = \frac{\mathrm{D} \cdot \mathrm{S}}{\mathrm{V} \cdot \mathrm{h}} (\mathrm{Cs} - \mathrm{Ct})$$

The dissolution rate (DR, dm/dt) is a function of the diffusion coefficient of the drug (D), the surface area of the drug (S), the effective (hydrodynamic) boundary layer thickness (h), the saturation solubility of the drug molecule (Cs), the concentration of the dissolved solute (Ct) and the volume of the dissolution medium (V). Highly permeable drugs like BCS class 1 and 2 would be absorbed quickly and therefore their concentrations of the dissolved solute (i.e. Ct) would stay low.

The pH of the GI fluids in the GI tract influences the dissolution rate of a drug. The pH influences the solubility of either acidic or basic drug, since ionized drugs tend to exhibit much greater aqueous solubility than unionized counterpart. Weakly basic drugs tend to have a slower dissolution rate at higher pH, whereas weakly acidic drugs dissolve faster at higher pH [39, 41].

In the previous research by Zhang et al. [20], dose number at neutral pH was used for the selection of drugs that gastric pH elevation potentially affects their drug exposure. That is, if the dose number for a test drug is larger than 1 and the test drug is a basic compound exhibiting pH-dependent solubility, there is a possibility that the drug exposure is decreased when it is co-administered with ARAs. However, the R^2 values for dose number (0.47 and 0.56) were smaller than those with solubility ratios of acidic pH to neutral pH (0.77 and 0.76). Solubility values at neutral and acidic pH would reflect the drug amount dissolved in the GI tract following oral administration with or

without ARAs, respectively. Therefore, the drug exposure ratios (with/without ARAs) would be related to the solubility ratios (acidic/neutral pH). Based on these results, the solubility ratios at acidic and neutral pH were more appropriate for predicting the effect of ARAs on drug exposure than dose number at neutral pH.

Tsume et al. [42] and Matsui et al. [43] developed multi-compartmental *in vitro* dissolution apparatus, Gastrointestinal Simulator (GIS) to predict *in vivo* dissolution from stomach to small intestine. They investigated the *in vivo* dissolution for dasatinib, fluconazole and dipyridamole under normal (pH 2.0) and elevated gastric pH (pH 6.0) by using GIS. The PK simulation results by GastroPlus™ software (Simulations Plus, Inc.) and the reduction ratios calculated by dissolved amount in GIS seemed to generally predict clinical DDI results with ARAs. However, this prediction would be time-consuming, because dissolution experiment with GIS system, measurement of drug concentrations and *in silico* simulation are required. Thus, for an early decision making of necessity and timing of a dedicated DDI study with ARAs, our simple prediction would be better, because only *in vitro* solubility data are required for estimating the effect of ARAs on drug exposure.

In drug development, it is important to identify factors affecting drug exposure. Since those factors have the potential to affect drug exposure, it may cause large inter-subject and intra-subject variability of drug exposure. Consequently, the variability makes study results of efficacy and safety complicated. Depending on exposure-response relationships for a test drug, drug companies have to consider setting dose modification rules in pivotal clinical trials and/or to prohibit co-administered drugs to secure efficacy and safety of patients. Also, assessment of those factors and description of the precaution in package insert would be required for appropriate use in clinical practice.

Thus, it is important to know factors affecting drug exposure in early stage of drug development.

Based on our finding, it is better to assess *in vitro* solubility values at both acidic and neutral pH and predict the effect of ARAs on drug exposure. With consideration for exposure-response relationship for a test drug, expected effect of ARAs on drug exposure, and prevalence of ARAs in the target disease, it is recommended to consider the necessity and timing of conducting a dedicated DDI study with ARAs. Although data used for this study is limited, drug exposure under the condition of being co-administered with ARAs is thought to be decreased by approximately 40%-50% as compared to that without ARAs when the solubility ratio is greater than 1000. Although it depends on the exposure-response relationships for the test drug, increase of dose level (e.g. 2-folds) may need to be considered to maintain efficacy by adjusting drug exposure.

In this research, solubility data of drugs at acidic and neutral pH available to the public were limited, although there were approximately 100 clinical DDI study data with ARAs. Also the pH values used for the assessment varied within a limited range among the test drugs due to limitation of available solubility data. In addition, although we excluded DDI study results investigating interaction between ARAs and test drugs via CYP enzymes and transporters, there might be unknown clearance mechanisms through CYP enzymes and transporters for the test drugs, and the interactions of metabolism and transporter might be included in the net effect of ARAs on drug exposure. Further investigation would be desirable to confirm the results from this research.

4. Overall discussion

Based on the research regarding factors affecting intra-subject variability, absolute oral BA was shown to be one of the major factors to predict the extent of intra-subject CV of drug exposure. Taking different strategies based on the absolute oral BA value is thought to be efficient. When absolute oral BA is less than 5%, the intra-subject variability is predicted to be greater than 30%. In this case, it is better to plan replicate crossover design and two-stage BE to mitigate the risk of BE failure. When absolute oral BA is greater than 80%, the intra-subject variability is predicted to be less than 20%. In the first BE study, intra-subject variability for a test drug is usually unknown. However, it would be efficient to estimate sample size assuming the intra-subject variability to be 20% for those drugs, instead of conducting a pilot BE study to know the intra-subject variability.

Acidic nature of drugs is also identified as an additional factor increasing intra-subject variability of Cmax as compared to AUC. Thus, when planning a BE study, it would be desirable to check whether a test drug is acidic compound with pKa less than 6. Considering these factors in drug development would be useful to mitigate risk of BE failure and lead to efficient drug development.

There is a possibility that ARAs affect drug exposure for orally-administered drugs having pH dependent solubility. Based on our research, solubility ratio of acidic pH to neutral pH is a potential factor relating to effect of ARA on drug exposure. Although the number of test drugs is limited, if the solubility ratio is greater than 1000, the effect of ARA on drug exposure is predicted to be about 40%-50%. For maintaining efficacy by adjusting drug exposure, increase of dose level (e.g. 2-folds) may need to be considered. Thus, investigating *in vitro* solubility at acidic and neutral pH is proposed in early stage

of drug development. If the solubility ratio is greater than 1000, it is recommended to conduct a dedicated DDI study with ARA in early stage of clinical development. If the solubility ratio is less than 10, the effect of ARA on drug exposure is predicted to be less than 10%. Thus, no dedicated DDI study with ARA is recommended. For other drugs of which solubility ratio ranges from 10 to 1000, necessity of conducting a dedicated DDI study with ARA would depend on exposure-response relationships for the test drug as well as the frequency of concomitant use of ARAs in the target disease. If the test drug has narrow therapeutic window, it is better to know the effect of ARA on drug exposure by conducting a dedicated DDI study or explore the effect by performing population PK analysis based on the information about co-administered drugs in clinical studies.

Based on this research, we found factors affecting drug exposure in BE studies and DDI studies with ARAs. Knowledge of these factors before conducting those clinical studies must be useful for establishing an appropriate study design, mitigating a risk of study failure, and judging the necessity and timing of such clinical studies. Thus, our findings will contribute to efficient clinical development of orally-administered drugs in the future.

5. Conclusion

Investigation of factors shown in this study (absolute bioavailability, acidic nature, and in vitro solubility) would help to predict the magnitude of effect on drug exposure. Since it leads to mitigating risk of BE failure, establishing an appropriate study design for BE studies and judging the necessity and timing of a DDI study with ARAs, our findings will contribute to efficient development of orally-administered drugs in the future.

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